

# A New Locus for Autosomal Recessive RP (*RP29*) Mapping to Chromosome 4q32-q34 in a Pakistani Family

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**PURPOSE.** To map the disease locus in a six-generation, consanguineous Pakistani family with autosomal recessive retinitis pigmentosa (arRP). All affected individuals had pigmentary retinopathy associated with symptoms of night blindness and the loss of peripheral visual fields by the age of 20 years, loss of central vision between the ages of 25 and 30 years, and complete blindness between the ages of 40 and 50 years.

**METHODS.** Genomic DNA from family members was typed for alleles at known polymorphic genetic markers using polymerase chain reaction. Alleles were assigned to individuals, which allowed calculation of LOD scores using the programs Cyrillic (<http://www.cyrillicsoftware.com>) and MLINK (Cherwell Scientific Publishing Ltd., Oxford, UK). The genes for membrane glycoprotein (*M6a*) and chloride channel 3 (*CLCN3*) were analyzed by direct sequencing for mutations.

**RESULTS.** A new locus for arRP (*RP29*) has been mapped to chromosome 4q32-q34. A maximum two-point LOD score of 3.76 was obtained for the marker D4S415, with no recombination. Two recombination events in the pedigree positioned this locus to a region flanked by markers D4S621 and D4S2417. A putative region of homozygosity by descent was observed between the loci D4S3035 and D4S2417, giving a probable disease interval of 4.6 cM. Mutation screening of two candidate genes, *M6a* and *CLCN3*, revealed no disease-associated mutations.

**CONCLUSIONS.** The results suggest that the arRP phenotype maps to a new locus and is due to a mutated gene within the 4q32-q34 chromosomal region. (*Invest Ophthalmol Vis Sci.* 2001;42:1436-1438)

Retinal photoreceptor dystrophies are a clinically and genetically heterogeneous group of retinal degenerations that together form the most frequent cause of inherited visual disorders, with an estimated prevalence of 1 in 4000.<sup>1,2</sup> Retinitis pigmentosa (RP) is characterized by progressive loss of vision, initially manifesting as night blindness and reduction in the peripheral visual field and later involving loss of central

vision.<sup>1</sup> Ophthalmoscopic examination typically reveals pigmentary disturbances of the midperipheral retina. RP may be inherited as an autosomal recessive, autosomal dominant, digenic, or X-linked trait. Autosomal recessive RP (arRP) accounts for approximately 20% of all cases of RP, whereas sporadic RP, which is presumed to be recessive in most cases, accounts for a further 50%.<sup>2</sup>

To date, approximately 17 loci for arRP have been reported.<sup>3</sup> In this study we identified a novel locus for arRP (*RP29*) on chromosome 4q32-q34.

## METHODS

The study protocol adhered to the tenets of the Declaration of Helsinki. We studied 16 members of a six-generation, consanguineous Pakistani family in which RP segregated as an autosomal recessive trait (Fig. 1). This pedigree contained six affected individuals in two branches. Fundus examination of all affected individuals revealed the typical clinical features of retinitis pigmentosa, including pigment deposition, attenuation of blood vessels, and pallid disc. The peripheral blood vessels were completely obliterated in individuals V:3, V:6, and VI:7 (Fig. 1). The early stage of the disease was clinically characterized by bone corpuscle-type pigmentation, deposited mainly in the equatorial region, and macular involvement in the later stages of the disease. Affected subjects experienced night blindness from the age of approximately 20 years and deterioration of visual acuity (central vision) between 25 and 30 years of age. By the age of 40 to 50 years affected subjects had no perception of light in either eye. Biomicroscopy showed the presence of cells in vitreous of individuals V:7 and VI:4. Anterior polar and posterior capsular lens opacities were also observed in individuals V:3 and V:6 (Fig. 1). Subjects were designated as unaffected if they showed no clinical evidence of RP by the age of 30 years.

## DNA Extraction

Peripheral blood samples were collected with informed consent from the affected and unaffected members of the family (Fig. 1). For comparison, 100 ethnically matched individuals with no personal or family history of retinopathy were selected to serve as control subjects. Genomic DNA was extracted from whole blood using an extraction kit (Nucleon II; Scotlab Bioscience, Strathclyde, Scotland, UK).

## Microsatellite and Linkage Analysis

Genomic DNA from family members was amplified by using primers for microsatellite markers for the known arRP loci<sup>3</sup> and whole-genome search<sup>4</sup> by polymerase chain reaction (PCR). PCR products were separated by nondenaturing polyacrylamide gel electrophoresis (Protogel; National Diagnostics, Manville, NJ) and visualized under UV illumination after staining with ethidium bromide. Alleles were assigned to individuals, and genotypic data were used to calculate the LOD scores using the programs Cyrillic and MLINK. Allele frequencies were calculated from the spouses in this family and a control ethnically matched population. The phenotype was analyzed as an autosomal recessive trait, with complete penetrance, and a frequency of 0.0001 for the affected allele.

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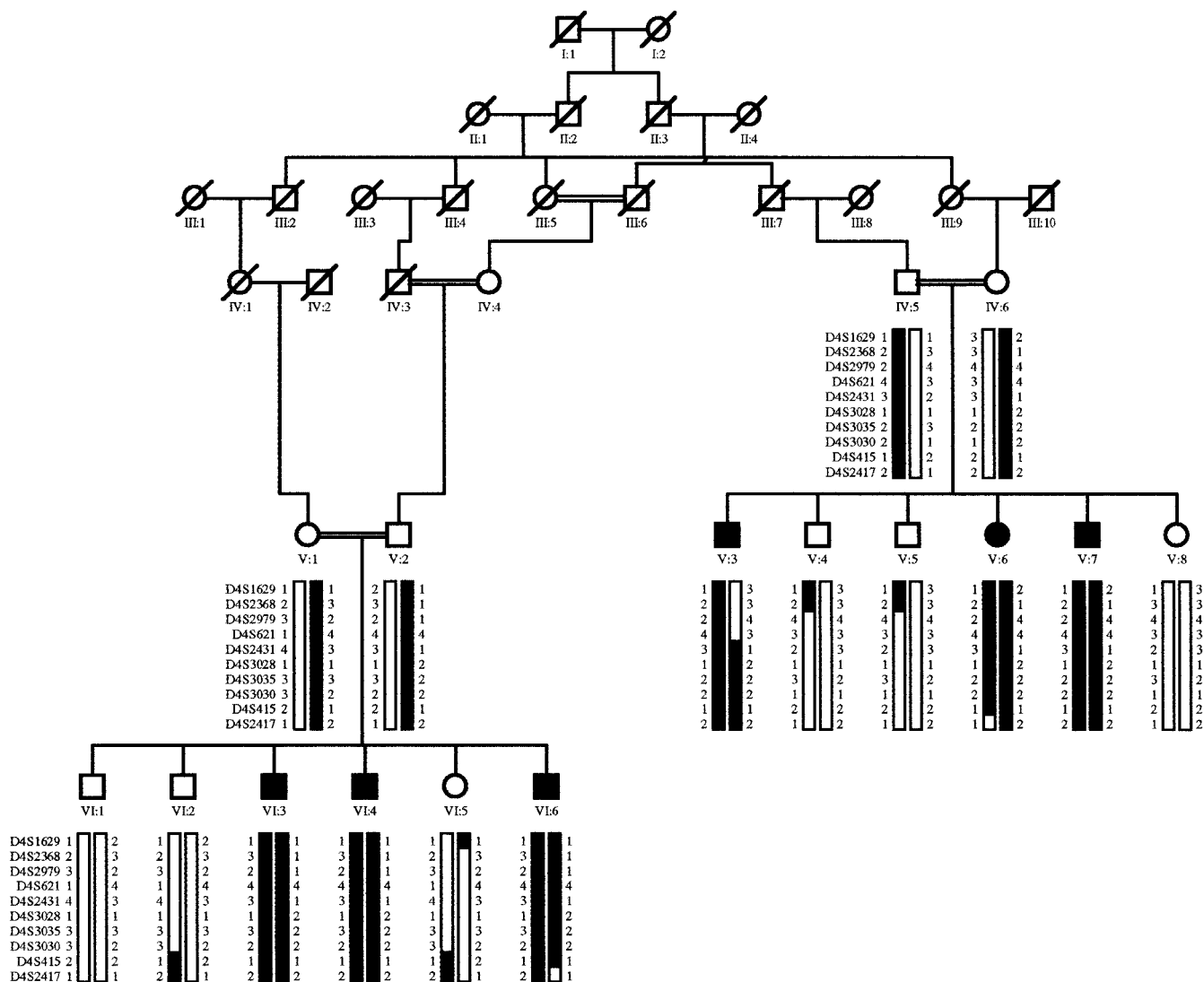


FIGURE 1. Pedigree of an arRP family with analysis of genotypic data for microsatellite markers. Circles: women; squares: men; filled symbols: affected individuals; open symbols: unaffected individuals. Double line connecting the spouses represents consanguinity.

### Mutation Screening

Intronic forward and reverse primers were designed for the exons of the candidate genes. The PCR reactions (25 or 50  $\mu$ l) were performed using DNA samples of unaffected parents for heteroduplex analysis under standard conditions with *Taq* DNA polymerase (Bio-Line; London, UK). Annealing was at the exon-specific temperature. To identify any heteroduplexes, the amplified exons were analyzed by electrophoresis using MDE gel (FMC, Rockland, ME).<sup>5</sup> The gels were run at 180 V overnight on a commercial apparatus (model 600S; Hoefer, San Francisco, CA). Products of PCR amplification were sequenced using a commercially available kit (Prism Ready Reaction Sequencing Kit; Perkin Elmer-ABI, Warrington, UK), and the products were analyzed on an automated sequencer (model 377; Perkin Elmer-ABI). All PCR products were sequenced in the forward and reverse directions.

### RESULTS

Linkage analysis was performed on this family as described earlier. The known arRP loci<sup>3</sup> were tested for linkage to the disease in this family using two microsatellite markers centered on the critical region of each locus. Linkage to any of the known arRP loci was not observed.

A genome-wide search was undertaken using 300 polymorphic markers spanning the entire human genome at approximately 20-cM intervals (Research Genetics, Huntsville, AL). Significant exclusion was obtained for all markers except those located on chromosome 4q32-q34. Haplotypes for these markers are shown in Figure 1. Two-point LOD scores between arRP and the markers in this region (D4S1629, D4S2368, D4S2979, D4S621, D4S2431, D4S3028, D4S3035, D4S3030, D4S415, and D4S2417) are summarized in Table 1. The maximum LOD score of 3.76 was obtained for the marker D4S415. A positive LOD score of 3.48 at  $\theta = 0.00$  was also obtained with D4S3030. Recombination events involving markers D4S621 in individual V:3 and D4S2417 in individuals V:6 and VI:6 define the centromeric and telomeric boundaries, respectively, of the 6-cM<sup>5</sup> disease locus between these markers (Fig. 1). Because this was a consanguineous family, a region of homozygosity would be expected to surround the associated gene. All the patients in both branches of this family were homozygous for alleles of the microsatellites D4S3030 and D4S415. This most probably indicates an area of homozygosity by descent, and it is therefore probable that the disease gene lies in this smaller interval, flanked by the markers D4S3035

TABLE 1. Two-Point LOD Score Data of an *arRP* Family for Chromosome 4q Microsatellite Markers

Marker	Recombination Fraction							$Z_{\max}$	$\theta_{\max}$
	0.0	0.01	0.05	0.1	0.2	0.3	0.4		
D4S1629	$\infty$	-1.54	-0.32	0.08	0.25	0.18	0.06	0.25	0.2
D4S2368	$\infty$	0.13	1.21	1.41	1.19	0.72	0.24	1.21	0.05
D4S2979	-2.89	-0.14	0.98	1.25	1.14	0.72	0.24	1.25	0.1
D4S621	$\infty$	1.61	2.04	1.99	1.56	0.98	0.39	2.04	0.05
D4S2431	0.72	2.13	2.52	2.44	1.89	1.15	0.39	2.52	0.05
D4S3028	-0.55	0.87	1.33	1.33	1.02	0.58	0.15	1.33	0.05
D4S3035	-0.54	0.89	1.35	1.37	1.08	0.65	0.22	1.37	0.1
D4S3030	3.48	3.41	3.13	2.76	2.00	1.21	0.46	3.48	0.0
<b>D4S415</b>	<b>3.76</b>	<b>3.68</b>	<b>3.36</b>	<b>2.95</b>	<b>2.09</b>	<b>1.20</b>	<b>0.38</b>	<b>3.76</b>	<b>0.0</b>
D4S2417	$\infty$	-0.91	0.24	0.53	0.53	0.33	0.12	0.53	0.1

$Z_{\max}$ , maximum LOD score;  $\theta_{\max}$ , recombination fraction of  $Z_{\max}$ . Line in bold indicates the marker with maximum LOD score.

and D4S2417. This interval spans only 4.6 cM (Fig. 1). These results have permitted us to identify a novel locus for arRP (*RP29*) at 4q32-q34. The locus does not overlap any previously identified retinal dystrophy locus; therefore, this represents the identification of a novel locus for a gene that could be important for normal retinal functioning. This 18th autosomal recessive locus illustrates genetic heterogeneity of the arRP phenotype.

The genetic databases<sup>6-8</sup> were searched to identify the candidate gene(s) in the region. The genes *M6a* and *CLCN3*, which were physically mapped within the disease region, were analyzed for mutation by heteroduplex analysis and direct sequencing. All the exons were amplified using primers designed for the intronic regions of the genes to permit detection of any splice site mutations. *M6a* is a cell surface glycoprotein, mainly expressed on neurons in the murine central nervous system (CNS), which plays significant roles in neural cell adhesion and some aspects of neurite growth.<sup>9-11</sup> *CLCN3* is a member of the voltage-gated chloride channel family and is expressed primarily in tissues derived from the neuroectoderm.<sup>12</sup>

Sequencing of both these genes revealed no disease-associated mutations. However, a T→G substitution was found in the noncoding region of exon 5 at nucleotide 1307 of the *M6a* gene.<sup>10</sup> No disease-associated segregation for this change was observed.

## DISCUSSION

Examination of the GeneMap'99<sup>6</sup> and analysis of the genomic sequence available from this region did not identify any candidates in addition to the two we have screened. The genomic sequencing of the region is unfinished as yet, and, in addition, many gaps remain that could explain the lack of identifiable candidates. Further isolation and characterization of novel transcripts from this region will aid the identification of this disease gene. Further effort is required to identify the disease-causing gene in this region of chromosome 4q, which is syntenic with

a region of mouse chromosome 8. There are, however, no reports of a mouse model with retinal degeneration mapping to this locus.

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